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SEPTAL PORES IN *TRICHOSPORON CUTANEUM*

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In strains of the yeast species *Trichosporon cutaneum* septal pores of the Basidiomycete-type were observed by electron microscopy.

Yeasts of the anascosporogenous genus *Trichosporon* are characterized by the formation of budding cells and septate mycelium which falls apart into arthrospores. A study by electron microscopy of reproduction in some species of this genus revealed the occurrence of septal pores in strains of *T. cutaneum*. In the present paper a description of the pores is given.

MATERIALS AND METHODS

Two strains were examined *viz*:

T. cutaneum (De Beurm., Gougerot et Vaucher) Ota, CBS 5597; and 5601.

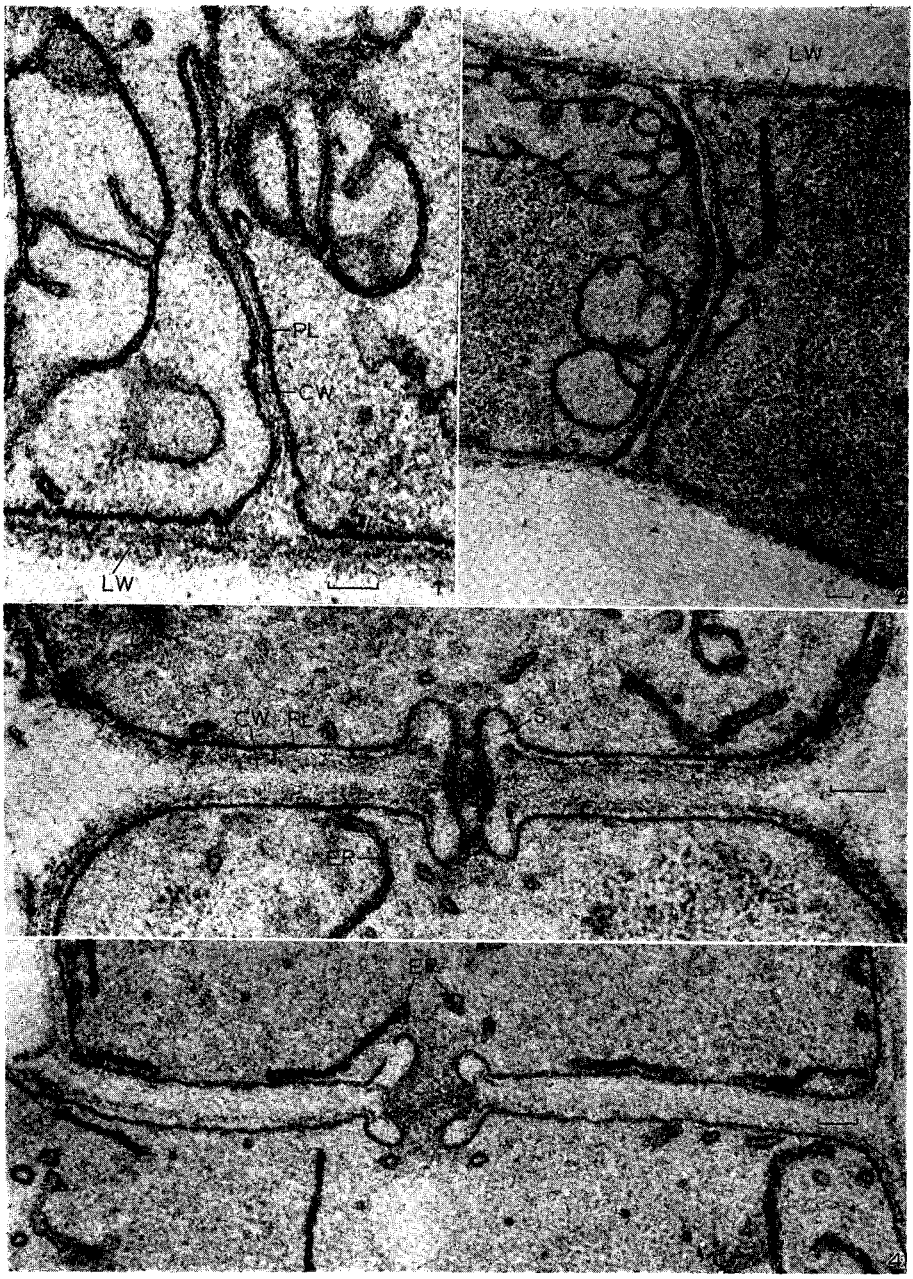
The yeasts were grown in shaken malt extract cultures for 16 or 24 hr. at 20°C. After collection and washing, the cells were fixed with 1·5% aqueous $KMnO_4$ for 20 min. at room temperature, washed with water, suspended in agar and dehydrated through a graded ethanol series. The cells were post-stained in a saturated solution of uranyl acetate in 100% ethanol for 1 hr. and embedded in Epon 812. After polymerization at 65°C, the material was cut on an LKB ultramicrotome with a diamond knife. Some of the sections were post-stained with lead citrate (Reynolds, 1963). Electron micrographs were taken with a Philips EM 300.

OBSERVATIONS

The strains had the morphological characteristics of the genus *Trichosporon*, *i.e.* budding cells and septate mycelium breaking up into arthrospores. Buds were generally formed on a broad base. In shake cultures in malt extract the strains exhibited chiefly mycelial hyphae and arthrospores, and only a few budding yeast cells.

The walls of yeast cells and hyphae showed thin layers of more and less electron-dense material (fig. 7). In the electron-dense layers 2 thin dark lines could be distinguished. The wall of the bud was continuous with the inner layer of the wall of the mother cell. The other layers of the latter were broken and formed a collar at the base of the bud. In yeast cells the number of layers increased with the age of the cells.

Different stages of cross wall formation in the hyphae were observed. From these observations, some of them in serial sections, we have deduced the following scheme of development.



Explanation of plates.
Symbols: CH = pore channel, CW = cross wall, ER = endoplasmic reticulum, L = plasmalemmasomes, LW = Lateral wall, P = plug, PL = plasmalemma, S = swelling.
The marker in figs. 1–5 represents 0·1 μ, in figs. 6–10: 0·5 μ.

Figure 1.—L. S. of hypha showing a partly formed cross wall. In the middle of the wall a light layer is visible.
Figure 2.—Section through a complete cross wall beside the pore.
Figure 3 and Figure 4.—Sections of hyphae through the cross wall with a pore, respectively with a narrow and a wide channel. In fig. 3 the cross wall has begun to split at the edge.

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Figure 5.—Section through an arthrospore with the plug still present. The plasmalemma follows the recess of the former swelling and partly surrounds the plug.
Figure 6.—Two separated arthrospores both showing the place of the former pore with swelling.
Figure 7.—Plasmalemmasomes in the cross wall. In the lateral wall lamellae are visible (arrowed).
Figure 8.—Plasmalemmasomes liberated upon splitting of the cross wall.
Figure 9.—T. S. through a dolipore showing in the centre a narrow channel. Plasmalemma and endoplasmic reticulum are present.
Figure 10.—Section through the lateral cell wall with a complex structure of double membranes.

The cross wall grows centripetally from the lateral wall as a thin plate consisting of a light layer between 2 slightly darker layers, surrounded by the plasmalemma (fig. 1, 2). The development is not always symmetrical. In the cross wall a central pore is left and around the pore the cross wall thickens. In longitudinal sections through the middle of the pore the thickened parts appear as 2 dumb-bells with a narrow channel between (fig. 3). However, the pore may also be wider in the middle (fig. 4). In older septa several layers of dark material separated by less electron-dense layers occur. In the swelling around the pore the dark layers diverge slightly. Most of the swelling is light. Around the pore, endoplasmic reticulum is present, presumably forming a pore cap.

In the formation of arthrospores the cross wall splits along the light inner layer, beginning at the edge (fig. 3) At this stage the material within the channel of the pore is often very dark This occlusion apparently plugs the pore. After completion of the splitting of the cross wall the plug, partly surrounded by a membrane which is probably the plasmalemma covering the pore wall, is still present (fig. 5); it later disappears In a young arthrospore the plasmalemma still follows the outline of the swelling around the pore, covering the original channel (fig. 6) The recess thus formed is filled with wall material of indistinct structure, and occasionally membranes

In hyphal cell walls 2 types of membranous structures were observed. The first type, considered to be plasmalemmasomes as defined by Marchant & Roberts (1968), had a unit membrane and occurred in the cross wall (fig. 7). The plasmalemmasomes were set free when the latter split up (fig. 8). The second type was found in the lateral wall and showed a complex structure of double membranes (fig. 10).

The structure of septum and septal pore in *T. cutaneum* resembles that of *Rhizoctonia solani* in the description by Bracker & Butler (1963). Both species have a cell wall in which several dark layers or lamellae may be observed. The septum has a light inner layer. The size of the dolipore, *i.e.* the pore surrounded by the swollen edge of the cross wall, in *Trich. cutaneum* is conspicuously small (0·3–0·45 μ in diameter) compared with that in *Rh. solani* (0·66 μ in young and 1·6 μ in the older cross wall). The pore cap in *T. cutaneum* is often scanty; it is formed by endoplasmic reticulum lying parallel to the cross wall and in the centre of the cell. Cortical endoplasmic reticulum, lying parallel to the lateral wall of yeast cells and hyphae, was scarce or absent.

A comparison of the dolipore in *T. cutaneum* with that in the ascosporogenous yeast *Endomycopsis platypodis* (Kreger-van Rij & Veenhuis, 1969a, b) shows distinct differences. The cross wall in *E. platypodis* consists of a light inner layer, primarily formed, between layers of darker material with which it later thickens. The swelling is also darker. Lamellae are not visible. In *T. cutaneum* the cross wall consists, from the beginning of its formation, of a light inner layer between 2 slightly darker layers. In older septa dark lamellae are present. The swelling is somewhat lighter. The dolipore in *E. platypodis* has no cap but it is plugged at both sides; it is bigger than that of *T. cutaneum*, namely about 0·7 μ.

Formation of arthrospores in *T. cutaneum* involves disintegration of the doli-

pore after occlusion of the pore channel. The occluding plug disappears. Observations suggest that it is removed from the wall either as a whole or split up. In the arthrospore a thickened part of the cell wall remains visible.

A sexual cycle for *T. cutaneum* is not known yet. The present observations of a septal pore of the Basidiomycete-type make it probable that this species is related to the Basidiomycetes. Moreover, the structure of the cell wall and the method of bud formation resembles those of yeasts of the basidiomycetous genera *Rhodosporidium* and *Leucosporidium*.

Résumé

L'étude au microscope électronique de la reproduction chez quelques espèces du genre *Trichosporon* a montré l'existence de pores de septum dans les souches de *Trichosporon cutaneum*. Ces pores sont du type de ceux des "Basidiomycète".

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